REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance, or in better condition for appeal.

Status of the Claims and Formal Matters

Claims 1-3 and 6-44 are currently pending in this application. Of these, claims 16-21, 23, and 35-44 were withdrawn from further consideration as allegedly being drawn to a non-elected invention. By this paper, Claims 1, 15, 22, and 34 have been amended, without prejudice and solely to expedite prosecution pursuant to the U.S. Patent & Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Claims 30 and 31 have been cancelled. Applicants assert the right to reclaim cancelled or withdrawn subject matter in co-pending applications. No new matter has been introduced by these amendments. Support for the amended recitations can be found throughout the specification as originally filed.

Rejections under 35 U.S.C. §103(a)

Claims 1-3, 6-14, 22, 24, 25, and 30-33 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths (U.S. Patent No. 20020119459, now U.S. Patent No. 6,808,882; "Griffiths") in view of Wangh et al (U.S. Patent No. 20040053254; "Wangh"). The Office Action contends that it would have been *prima facie* obvious to one of ordinary skill in the art to combine the method of Griffiths for amplifying nucleic acids in a microcapsule, such as a water-in-oil emulsion, on the surface of a bead with the method of Wangh for non-symmetric PCR using primers at unequal concentrations, since this method, which causes one of the primers to be used to exhaustion, is highly suited for amplifications that utilize small reaction volumes and very low copy numbers of target sequences. The Office Action further states that the assay may be performed in which either of the primers may be attached to a bead, and can be used in the microcapsule format allegedly taught by Griffiths. The Office Action also argues that it would have been obvious to use emulsion droplets of larger sizes such as in the range of 50 μm or in the range of 10 μm as allegedly used by Griffiths, since these differences in emulsion droplet size would not be expected to greatly alter the conditions for amplification, and a skilled

artisan would allegedly recognize that the droplet sizes can be adjusted to maximize the desired results. Applicants respectfully disagree and traverse this rejection in view of the amendments to the claims and remarks presented herewith.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. MPEP §2142. Applicants respectfully contend that in view of the amendments to the claims and remarks presented herewith, the combination of Griffiths and Wangh cannot be used in support of an obviousness rejection under §103(a), because Griffiths and Wangh do not teach or suggest all of the instant claim limitations.

The instant invention relates to a method of independent and parallel production of immobilized populations of amplified nucleic acid copies, wherein each population is derived from one single stranded template nucleic acid. The problem solved by the present invention is a method to efficiently amplify individual copies of a template nucleic acid, and drive the amplified copies to bind to a solid substrate. A key feature of this problem is that amplifying a nucleic acid in solution, particularly the kinetics of annealing a primer to a template, is much more efficient than amplifying nucleic acids in solid phase, especially as the length of the nucleic acid increases. See, for example, page 6, lines 11-13 of the instant specification as originally filed. A second and even more important aspect involves driving the amplified copies to bind to the solid substrate in a compartmentalized environment (such as a microreactor comprised of a water-in-oil emulsion) so that the concentration of the bound copies is high. Applicants respectfully contend that establishing and maintaining a segregated environment of discrete populations of single stranded nucleic acid template molecules, efficiently amplifying each template in the segregated environment so that products from different amplification reactions do not mix, and immobilizing the amplified copies to a substrate to maintain the segregation in a way that can be easily manipulated for other processes, such as sequencing, are all objects of the

present invention that are not taught, suggested, contemplated or envisaged by the cited references Griffith or Wangh.

The solution provided by the presently claimed invention takes advantage of limiting concentrations of primers that are used in an asymmetric nucleic acid amplification reaction to drive the amplified products to bind to a population of primers that are bound to a solid substrate during the reaction. Importantly, the presently claimed invention includes two populations of a first primer species: a first population of the first primer species in solution phase in a "limiting" concentration relative to a second primer species that is also in solution phase and which binds to the complementary strand of the nucleic acid template, and a second population of the first primer species attached to a solid phase. During amplification of the single stranded nucleic acid template, the solution phase population of the first primer species becomes depleted, while the second primer species in solution is present in excess and continues to generate amplified copies of the complementary strand of the nucleic acid template. The amplified nucleic acid template strands are driven to bind to the first primer species that are attached to the solid phase, because the number of amplified copies of the template strands in solution phase is substantially greater than the concentration of complementary strands, due to depletion of the solution phase population of the first primer species. Thus, the only available binding partner for the amplified copies in solution is the second population of the first primer species bound to the solid phase, essentially "forcing" the amplified copies to bind to the first primer species on the bead.

Griffiths or Wangh, whether considered alone or in combination with each other, fail to describe or appreciate the problem solved by the present invention. Neither Griffiths nor Wangh teach or suggest the use of primer depletion in an asymmetric amplification reaction to promote binding of a selectively amplified template to a solid substrate. Neither Griffiths nor Wangh disclose the use of three primer populations to carry out asymmetric amplification of a single stranded nucleic acid template resulting in amplified products that are bound to a solid support within an aqueous microreactor and segregated from other amplification reactions occurring in parallel.

Griffiths relates to a method for isolating one or more genetic elements encoding a gene

product having a desired activity, comprising compartmentalizing the genetic elements into microcapsules, expressing the genetic elements to produce their respective gene products inside the microcapsules, sorting the genetic elements that produce the gene product having the desired activity *via* a change in the optical properties of the genetic elements. Griffiths does not teach or suggest performing asymmetric PCR using three populations of primers: a first population of a first primer that is bound to a solid support, a second population of the first primer that is present in the amplification reaction solution, and a population of a second primer that is present in the amplification reaction solution in excess of the first primer. Notably, Griffiths is silent regarding asymmetric PCR in general.

Wangh does not cure the defects of Griffiths. Wangh describes a non-symmetric method of amplifying nucleic acids that uses two populations of primer molecules: one "Limiting" primer that is present in low concentrations, and one "Excess" primer that is present in higher concentrations relative to the "Limiting" primer. Each primer is specifically designed to have different melting temperatures, and the Wangh method requires temperature modulation after a certain number of cycles (e.g., after the Limiting primer has been depleted) to allow the Excess primer to amplify efficiently. Wangh, however, is silent regarding three populations of primers as described by the instantly claimed invention. In particular, Wangh does not teach or suggest one population of one primer species that is limiting in solution phase and a second population of the same primer that is bound to a solid phase. Wangh is also silent regarding the advantage of driving nucleic acids to bind and amplify on a bead using two populations of the same primer. Rather, Wangh describes asymmetric amplification in solution phase and in solid phase as <u>two separate embodiments</u>, wherein the solid phase embodiment requires that there are no solution phase populations of the species that is bound to a bead, and wherein the solution phase embodiment does not use a solid phase support.

<u>Wangh's Solution-Phase Embodiment</u>

Wangh also describes a solution-phase embodiment, wherein Wangh's "LATE-PCR" method is used without a solid phase support. In this embodiment, asymmetric PCR occurs solely in solution phase, whereby the Limiting primer species is present in solution at low

concentration, and the Excess primer species is present in solution at a higher concentration relative to the Limiting primer. According to the Wangh method, the initial amplification cycles result in double-stranded product, but after the Limiting primer is depleted from the solution, one strand is preferentially amplified from the Excess primer remaining in solution. See, for example, Wangh at page 5, paragraph [0025], where Wangh discloses:

> In the early cycles of a LATE-PCR amplification, when both primers are present, LATE-PCR amplification amplifies both strands of a target sequence exponentially, as occurs in conventional symmetric PCR. LATE-PCR then switches to synthesis of only one strand of the target sequence for additional cycles of amplification.

This embodiment of the Wangh method is clearly different from the instantly claimed methods. The solution-phase embodiment described by Wangh does not use a solid-phase support, such as a bead.

Wangh's Solid-Phase Embodiment

See, for example, Wangh at paragraph [0181], where Wangh states that,

> In another embodiment of the invention, the LATE-PCR can be carried out under conditions in which one of the primers, most preferably the Excess Primer, is fixed to a solid matrix or surface such that each cycle of primer extension results in construction of an extended primer strand which remains attached to the solid surface, for example, a bead or the wall of the reaction chamber. It is anticipated that under these conditions $T_{m[0]}$ of the attached primer will be additionally dependent on the fact that the primer is not freely diffusible, as well as by the packing density of the primer on the surface, by the volume, and by the geometry of the space in which the reaction takes place. Therefore, the $T_{m[0]}$ of the primer,

for instant $T_{m[0]}^{X}$, will have to be determined empirically under the experimental conditions of the reaction.

Wangh does not describe binding the asymmetrically amplified nucleic acid strands that are synthesized by the Excess primer species in solution to the second population of the first primer species attached to the solid phase. Rather, as described by Wangh above, the binding of nucleic acid strands to a solid substrate occurs during the asymmetric amplification process and not binding of the product of the asymmetric amplification process as claimed by the present invention.

Wangh describes that the "Excess Primer" is present in high concentrations in the solid phase, while the "Limiting Primer" is present in low concentrations in the solution phase. The presently claimed invention, on the other hand, describes the solution phase first primer species and the solid phase first primer species that are at concentrations lower than the second primer species. Applying Wangh's method, the limiting primer must be depleted before the excess primer can preferentially amplify the target strand. Thus, in the context of the present invention, Wangh teaches that the solution phase primers must be depleted before binding to the beads and bead-bound amplification can occur. This, however, is not what occurs in the practice of the present invention.

Unlike Wangh, the presently claimed asymmetric PCR invention can be used to achieve amplification of nucleic acids on a solid support by first depleting the low concentrations of a first primer species in solution and thereafter forcing the amplified product to bind to the first primer species in solid phase. In contrast to Wangh, the second primer species is present in higher concentrations relative to the first primer species and remains present in solution phase even after the solution phase first primer species has been depleted and the desired amplified copies are bound to the first primer species in solid phase. Wangh simply teaches a method of selective amplification of one nucleic acid strand using two populations of primers: one population that is used to exhaustion and one population that is present in high concentrations relative to the other. Applying the Wangh method, only after one primer population is depleted, can the other primer population in excess continue to mediate amplification of the other strand.

According to Wangh's teachings, it would be unlikely that selective amplification would occur on the bead if the second primer species were still present in solution. Therefore, Wangh teaches away from the present invention.

It is notable that neither the solution-phase embodiment nor the solid-phase embodiment of Wangh specify that a single-stranded template is preferentially driven to bind to a solid phase support, where a primer population remains present in the solution phase. In contrast, this preferential binding of a single-stranded template of interest to the solid phase, even in the presence of primers remaining in solution, is a key aspect of the instant invention. In essence, Wangh teaches away from the present invention, because the Wangh disclosure teaches that the reaction must either take place in solution phase, **or** can occur in solid phase so long as the primer in solution is entirely depleted, neither of which is practiced by the instantly claimed invention. There is no teaching, suggestion, or disclosure whatsoever that the solid-phase embodiment and the solution phase embodiment of Wangh can be combined. In the absence of such disclosure, the skilled artisan would not arrive at the present invention, especially in view of knowledge in the art that amplification reactions in solid phase occur much less efficiently than amplification reactions occurring in solution, and further validated by the teaching in Wangh that one can perform asymmetric PCR in solution phase **or** in solid phase, but not a combination of the two.

For at least all of the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejections over Griffiths in view of Wangh.

Claims 15 and 26-29 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Wangh and further in view of Jurinke et al (U.S. Patent No. 6,303,309). The Office Action states that although neither Griffiths nor Wangh teach a method for amplifying one or more nucleic acids wherein at least 1,000,000 amplification copies of each target nucleic acid molecule are bound to each bead, or where the beads are Sepharose beads, it would allegedly have been *prima facie* obvious to the skilled artisan to combine the methods of Griffiths and Wangh for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using non-symmetric PCR with that of Jurinke for purification of

PCR products using solid supports such as magnetic or Sepharose beads, since the use of such beads allows further purification and extensive washing to remove all excessive reaction components prior to final recovery of the final PCR product. According to the Office Action, the ordinarily skilled artisan would have been motivated to use magnetic or Sepharose beads as allegedly taught by Jurinke for binding and purifying PCR or other amplification products generated in a microreactor, since these beads have a large capacity and high affinity for such products, especially when using highly stable binding pairs such as biotin and streptavidin to form complexes of the amplification products on the beads. Applicants disagree and traverse this rejection in view of the amendments to the claims and remarks herewith.

For reasons discussed herein, neither Griffiths nor Wangh teach each and every limitation of the instant claims, as required by §103(a). Jurinke fails to cure the deficiencies of Griffiths and Wangh, because Jurinke merely relates to a method for dissociating biotin complexes comprising a biotin compound and a biotin-binding compound. The Jurinke method involves contacting the complex with an effective amount of an amine compound, which causes the complex to dissociate. Jurinke does not teach, suggest, or disclose methods of amplifying nucleic acids on a solid phase support such as a bead, using two populations of a first primer that are present in each of a solution phase and in a solid phase, where the two populations are present in concentrations lower than the concentration of a second primer in solution phase. Because Jurinke is silent regarding methods of asymmetrically amplifying nucleic acids, Applicants respectfully contend that the combination of Griffiths, Wangh, and Jurinke do not teach or suggest all of the instant claim limitations and thus, cannot be used in support of an obviousness rejection under §103(a). Reconsideration and withdrawal of the §103(a) rejection over Griffiths in view of Wangh and further in view of Jurinke are respectfully requested.

Claim 34 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Wangh and further in view of Nakano et al (J. Biotech. (2003) 102: 117-124). The Office Action contends that it would have been *prima facie* obvious to the skilled artisan to combine the methods of Griffiths and Wangh for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using a non-symmetric PCR with that of

Nakano, since Nakano allegedly teaches a method for amplifying multiple nucleic acids in water-in-oil emulsion that is adaptable to the methods of Griffiths and Wangh using a bead to bind amplification products in the emulsion. The Office Action further argues that an ordinary practitioner would allegedly have been motivated to use the system of Griffiths and Wangh for amplifying multiple nucleic acid targets of different sizes in an emulsion containing a bead using a non-symmetric PCR process, since the products allegedly can be easily purified simultaneously on the same bead and later separated by sizing methods or by gel electrophoresis. Applicants traverse this rejection in view of the amendments to the claims and remarks presented herewith.

In view of the deficiencies set forth above, the combination of Griffiths and Wangh do not result in the instantly claimed invention as claimed, nor does it result in an obvious variant of the instant invention. Griffiths and Wangh do not teach or suggest all of the instant claim limitations, namely the requirement of two populations of a first primer species that is present in a solution phase and in a solid phase, wherein both populations of the first primer species are in limiting concentrations relative to a second primer species that is present in the solution phase. Griffiths and Wangh, alone or in combination, do not teach a method of amplifying a nucleic acid on a bead under these primer concentrations. Indeed, the combination of Griffiths and Wangh teach away from the instantly claimed invention, because Wangh in particular suggests that amplification of a nucleic acid template on a solid support requires the depletion of the solution phase primer and the presence of a high concentration of Excess Primer on that solid support. The present invention, in contrast, is a surprising, non-obvious discovery that amplification of a nucleic acid of interest can be driven to occur on the surface of a bead, even though the primer concentrations on the bead are limiting with respect to the primer concentrations of the second primer species in solution.

Nakano relates to single-molecule PCR using a water-in-oil emulsion, wherein limiting dilutions of the DNA template were used to create conditions where one molecule of template was amplified inside a droplet comprised of silicone oil, Triton X-100, and PCR buffer. Nakano does not remedy the defects of Griffiths and Wangh, because Nakano does not teach, suggest, or disclose PCR that uses two primers that are of unequal concentrations such that one strand of

double-stranded template DNA is preferentially amplified over the other strand. Nakano is also silent regarding a method of amplifying a nucleic acid template on a bead, using two populations of a first primer species - one that is retained in the solution phase and one that is present on the surface of the bead, and a population of a second primer species that is present in solution phase in concentrations greater than either of the two populations of the first primer species. Nakano is also deficient because Nakano fails to teach or suggest amplification reactions in the presence of a solid phase support.

Consequently, in view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejections over Griffiths in view of Wangh and further in view of Nakano.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Respectfully submitted,

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